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Prior studies in the developing chick embryo indicate that Hoxd10 and Hoxd11 have opposing effects on the specification of motoneuron subtypes within the lateral motor column (LMC) of the lumbosacral (LS) spinal cord. Hoxd10 is initially expressed by newly differentiated motoneurons in all LS segments but later restricted to rostral LS motoneurons (~LS1–5). Hoxd11, in contrast, is expressed only in caudal LS segments (~LS4–8). When overexpressed in LS segments, Hoxd10 promotes the development of motoneurons bearing molecular markers and projection patterns characteristic of lateral LMC (LMCl), while Hoxd11 suppresses LMCl development. These effects mirror normal rostro-caudal differences in subtype distribution. Hoxd11 also appears to regulate the extent of the LS LMC as a whole by direct or indirect downregulation of Foxp1, a transcription factor critical for LMC development, and upregulation of two factors that define the medial motor column (MMC), Lim3 and Scip. To elucidate mechanisms of Hox action, we created a hybrid protein in which the DNA-binding homeodomain of Hoxd10 was replaced with that of Hoxd11 (Hoxd10^{d11HD}). Hoxd10^{d11HD}, when expressed in rostral LS segments, behaves in a manner similar to Hoxd11, and in direct opposition to Hoxd10, by suppressing development of the LMCl. However, it does not appear to affect total LMC size. We therefore propose that the repressive effects of Hoxd11 on LMCl formation are mediated primarily by its homeodomain, and that the homeodomain is sufficient to direct some but not all Hoxd11 actions.

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Program/Abstract # 487
Sensory neurons are required for migration and axon pathfinding of relay motor neurons

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Wiring the billions of neurons in the vertebrate central and peripheral nervous systems into functional circuits is one of the most complex processes in developmental neurobiology. A major challenge is to understand the logic underlying the assembly of neurons into functional circuits. Cell migration and axon pathfinding are critical patterning events that contribute to the assembly of neural circuits, but how these events are coordinated remains unclear. In the vertebrate head, we show that epibranchial placode-derived sensory neurons act as an intermediate target that coordinates the migration and axon pathfinding of parasympathetic relay motor neurons along the rostrocaudal axis of the body. In the absence of placodal sensory neurons, migratory neural crest destined for the postganglionic motor neuron fate undergo programmed cell death and axons of preganglionic motor neurons terminate abruptly in the area normally occupied by placodal sensory neurons, thereby failing to reach their distant target sites. Placodal sensory neurons are thus required for patterning the stereotypic relationship of relay motor neurons, presaging their ultimate integration into the sensory pathway of the parasympathetic reflex circuit.

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Program/Abstract # 488
The role of Tgif and Tgif2 during head development

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Holoprosencephaly (HPE) is the most common forebrain malformation in humans. Tgif (TG-interacting factor) and Tgif2 encode transcriptional repressors that regulate the TGFb pathway via direct association with Smad proteins. In humans, loss-of-function mutations in the TGIF gene cause HPE. During mouse embryonic development, both Tgif and Tgif2 are widely expressed suggesting possible functional redundancy. Tgif;Tgif2 double knock-out mouse embryos fail to gastrulate due to the ectopic upregulation of Nodal pathway. To dissect the possible role of Tgif and Tgif2 during the development of mouse embryo proper, we generated mice with epiblast specific deletion of Tgif and null alleles of Tgif2. *Sox2Cre;Tgif$^{f/f}$;Tgif2$^{-/-}$* (Tgif;Tgif2cdko) embryos have defects of left–right patterning and anterior head structure. In the mutant embryos, the situs specific molecular markers, such as Nodal and Pitx2, are expressed bilaterally. Intriguingly, the phenotype was partially rescued in Nodal$^{lacZ/+}$;Tgif;Tgif2cdko embryos when the dose of Nodal was genetically reduced. Importantly, Tgif;Tgif2cdkomutant embryos have HPE. Scanning EM analysis shows that Tgif;Tgif2cdko embryos lack the separation of rostroventral neural tissue at E9.25. Molecular analysis for Shh and Fgf8 mRNA shows that rostroventral forebrain tissue formation is defective. These results suggest that the patterning of rostroventral brain tissue is impaired. Taken together, these results indicate that Tgif and Tgif2 have significant roles during the patterning of neural tissue, presumably by regulating a TGFb signaling pathway.

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Program/Abstract # 489
Mesodermal Wnt4a signaling regulates segmentation of head mesoderm and pharyngeal endoderm in zebrafish

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All vertebrates have a unique segmented structure in the developing head — the pharyngeal arches. The pharyngeal arches are composed of all embryonic germ layers. Whereas pharyngeal endoderm (PE) segmentation is essential for segmentation of other tissues in the pharyngeal arches, little is known about the tissue interactions and molecular pathways that drive PE segmentation. Using transgenic imaging approaches in zebrafish, we show intimate interactions of the lateral plate mesoderm (LPM) and PE during head segmentation. Next we show that Wnt4a controls segmentation of both the LPM and PE. *wnt4a* is expressed in the LPM directly adjacent to the PE just before and during PE segmentation. Reducing Wnt4a levels using a Wnt4a-morpholino (MO) blocks segmentation of the LPM and PE. Moreover, we use the UAS/Gal4 system to manipulate Wnt signaling in specific tissues and show that inhibition of canonical Wnt signaling in LPM, but not PE, phenocopies the segmentation defects of Wnt4a-MO animals. In addition, transgenic expression of Wnt4a in LPM, but not PE, partially rescues the LPM and PE segmentation defects of Wnt4a-MO animals. Finally, nitroreductase-mediated LPM ablation also causes defects of PE segmentation. All together, these data show that 1.) mesodermal Wnt4a signaling is required autonomously for LPM segmentation and 2.) segmentation of LPM is essential for PE segmentation. In conclusion, our study reveals an unappreciated role of LPM in the initial establishment of vertebrate head segmentation.

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Program/Abstract # 490
Notch and Fgf signaling patterns the vertebrate dorsal face

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The development of the face depends on the regionalization of neural crest precursors into distinct dorsal and ventral domains. Previous research has shown that Endothelin 1 (Edn1) is required for patterning the ventral face, in part by regulating *Dlx* expression; however, little is known about the factors required for development of the dorsal face. We have recently identified a mutation in the zebrafish gene *jag1b* that results in dorsal-specific defects. In *jag1b* mutants, we observe an expansion of *Dlx* genes into the dorsal domain. Jagged is one class of ligands for Notch receptors. Here we show that overactivation of the Notch pathway results in loss of ventral genes and corresponding defects in the ventral facial skeleton, further supporting a role of Jagged-Notch signaling in promoting dorsal facial identity. In addition, we find an autoregulatory loop of *jag1b* and *notch2* expression that propagates in a dorsal to ventral wave of Notch activity during facial development. Based on these results, we propose a model in which dorsal–ventral facial identities are specified by dynamic interactions between a wave of Notch signaling arising dorsally and a gradient of Edn1 signaling arising ventrally. In our model, expression of *jag1b* localizes Notch activity to dorsal skeletal precursors. How then is *jag1b* expression established dorsally? Here we show that the endoderm is required for *jag1b* expression. Moreover, as the endoderm expresses *Fgfs*, we next investigated the role of Fgf signaling in patterning. Using a transgenic to block Fgf signaling at patterning stages, we observe a transformation of dorsal structures to a ventral identity, a defect similar to that observed in *jag1b* mutants.

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Program/Abstract # 491

Hand2 loss leads to aglossia from failure to repress Dlx5/6

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Lower jaw development is a complex process orchestrated by signaling cascades that are regulated temporospatially and are constantly refined through permissive and inhibitory signals. We have previously shown that endothelin-A receptor (Ednra) signaling is crucial for establishing the identity of cranial neural crest cells (NCCs) in the mandibular pharyngeal arch through a mechanism that involves Dlx5 and Dlx6. Dlx5/6 in turn induce expression of the gene encoding the basic helix–loop–helix transcription factor Hand2. While this pathway places Hand2 at the center of a complex signaling cascade, little is known about the function of Hand2 in mammalian facial development because *Hand2*$^{-/-}$ embryos die by embryonic day (E) 10.5 from vascular failure. To circumvent this lethality, we created a conditional targeted *Hand2* mouse line using a Cre-*lox*P approach. Using the *Wnt1-Cre* mouse line, we selectively deleted *Hand2* within all NCCs. We find that *Hand2* conditional knockout mice exhibit facial defects that include mandibular hypoplasia and absence of the tongue (aglossia). The aglossia is preceded by aberrant maintenance of *Dlx5* expression in the disto-oral mandibular arch mesenchyme. In vitro studies show that Hand2 represses the Dlx5/6 pharyngeal arch-specific enhancer. Together, these data suggest that Hand2 normally ensures normal tongue development by repressing *Dlx5/6* expression within the disto-oral mandibular arch. In the absence of Hand2, *Dlx5/6* expression is maintained and ectopically activates an osteogenic program at the expense of a tongue development program.

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Program/Abstract # 492

prdm1 is required for zebrafish craniofacial development

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Defects in neural crest cell (ncc) differentiation result in many human congenital birth defects. A better understanding of cell fate determination, migration, and differentiation of nccs during formation of the craniofacial skeleton is an important step towards developing approaches to prevent and repair human ncc-associated birth defects. We focused on the role of the transcription factor *prdm1* in the differentiation of nccs into the craniofacial skeleton. It does not appear that *prdm1* is required for ncc migration but beginning at 16 hpf and continuing through 56 hpf, it is expressed in the correct temporal and spatial pattern to be involved in the differentiation of craniofacial structures. During early craniofacial development, *prdm1* is expressed in the pharyngeal arch region and later appears to be expressed in an endodermal pouch, the otic vesicle, and pharyngeal teeth. A loss of *prdm1* results in defects in posterior pharyngeal arches including a loss of cartilage and dermal bone. A reduction in Fgf and retinoic acid signaling alters *prdm1* expression suggesting that *prdm1* is operating via these signaling pathways to pattern the posterior craniofacial skeleton. Finally, loss of *prdm1* results in a reduction in proliferating cells suggesting that the cartilage defects may be due to a reduction in the number of nccs. Future experiments will further determine the interactions between *prdm1* and other known craniofacial genes as well as the function and tissue specific requirement for *prdm1* during craniofacial development.

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Program/Abstract # 493

RA-Noggin beads induce TBX22, a frontonasal mass-specific gene in the maxillary prominence, however over-expression causes clefting rather than a transformation in identity

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Previous data from our lab has shown that RA and Noggin, when applied to the early avian face, can transform the maxillary prominence to a frontonasal mass. Here we use Affymetrix microarrays to determine the transcriptional consequences, 16 h after implantation of RA-Noggin beads. We also compared expression to stage-matched normal stage 17 embryos to determine whether frontonasal mass-specific genes were induced. Chip-wide analysis revealed that the RA-noggin samples clustered together but were distinct from either the frontonasal mass or maxillary prominence. RA-noggin induced several genes that are highly expressed in the normal frontonasal mass including *TBX22, SOX8*, *ALX1*, and *OSF2*. Validation with QPCR and wholemount in situ hybridization confirmed that *TBX22* was ectopically expressed in the RA-Noggin treated maxillary prominence and that Noggin induced expression to a greater extent than RA. We then tested the effects of ectopic expression of *TBX22* using retroviruses and found that instead of inducing frontonasal mass characters such as ectopic egg teeth or cartilages, facial clefting